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Volume I

DEVELOPMENT AND VALIDATION OF METHODS FOR APPLYING PHARMACOKINETIC DATA IN RISK ASSESSMENT

VOLUME I: EXECUTIVE SUMMARY/ INTRODUCTION

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Maj, USAF, BSC
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Physiologically-based pharmacokinetic (PBPK) Modeling as a risk assessment tool is sensitive to uncertainties and variation in model parameters. This volume summarizes the approaches taken to (1) describe the construction of PBPK models for trichloroethylene, tetrachloroethylene, methylchloroform and vinyl chloride and (2) study the uncertainty associated with PBPK models and model parameters. Also discussed in this volume is the methodology involved with risk assessment software development and execution of a sensitivity/uncertainty analysis.

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FOREWORD

This report has been prepared by Clement International Corporation, K.S. Crump Division, for the Department of the Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base in response to a request to investigate the incorporation of pharmacokinetic modeling into quantitative risk assessment. This report contains the results of this multiyear effort and reflects the changes in direction and priorities as this project has evolved. The Project Director was Dr. Kenny Crump and the Principal Investigator for this project was Mr. Bruce Allen; other investigators who provided technical support and internal peer review were Drs. Crump and Annette Shipp. Mr. Allen was assisted in the pharmacokinetic modeling and analyses primarily by Mr. Christopher Rambin and by Ms. Robinan Gentry. The sensitivity analyses were conducted by Mr. David Farrar, Dr. Crump, Dr. Richard Howe, and Mr. Allen. The software was developed by Ms. Cynthia Van Landingham, Mr. William Fuller, Mr. Eric Brooks, Dr. Howe, and Mr. Allen. The authors wish to acknowledge the support provided by Dr. Jeffery Fisher and Lt. Col. Harvey Clewell, who are at the Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base, and Drs. Melvin Andersen and Michael Gargas, formerly with the Harry G. Armstrong Aerospace Medical Research Laboratory and now with CIIT.

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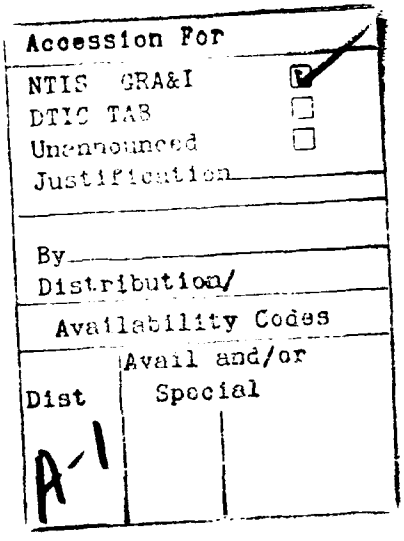

EXECUTIVE SUMMARY

This report presents the results of a multiyear study designed to examine pharmacokinetic modeling in the context of risk assessment. The project was begun by conducting a survey of the literature on existing physiologically based pharmacokinetic (PBPK) models and the experimental pharmacokinetic data for selected volatile organic chemicals. Following this survey, we investigated models for tetrachloroethylene (discussed in Volume III, Part 1) and identified some of the issues related to the use of PBPK modeling in risk assessment. Our efforts to resolve these issues continued along two related lines of investigation: 1) construction of PBPK models of selected volatile organic chemicals, trichloroethylene, tetrachloroethylene, methyl chloroform, and vinyl chloride; and 2) formal study of uncertainty associated with PBPK models and model parameters.

The first line of investigation, PBPK modeling for volatile organics, led to development of models of increasing complexity. The models included simulation of the kinetics of primary metabolites and in the case of vinyl chloride, modeling of the depletion of glutathione, which is essential to a key secondary metabolic pathway. These models were constructed to simulate, pharmacokinetically, experimental rodent studies and potential human exposures. From those simulations, we determined estimates of lifetime human cancer risk based on the pharmacokinetically derived delivered doses (dose surrogates) in target tissues.

The second line of investigation led to clarification of the issue of model uncertainty. Approaches for assessing sensitivity and for determining

the effects of parameter uncertainty were developed. In addition, we developed a computer software package (PBPK_SIM) for uncertainty analyses.

The project reported here has spanned almost four calendar years. As a result, the specific topics and focus of the work have evolved and have lead to some changes in emphasis and in deliverables desired by the supervising Air Force personnel. In part, this has occurred because of changes in personnel and shifts in priorities. Nevertheless, this report reflects all of the work that has been completed over the course of this contract.

ISSUES IN PBPK MODELING: Preliminary consideration was directed toward identification of the issues related to the use of PBPK modeling for risk assessment purposes. The issues included:

- How PBPK model output could be used for other aspects of a risk assessment, particularly dose-response modeling.
- What type of PBPK model output would be most useful for risk assessment.
- What uncertainties are associated with risk assessment and what impact does PBPK modeling have on risk assessment uncertainty.

These issues are discussed further in the general introduction to this document (Volume I, Part 1). Emphasis is placed on the uncertainties, which were categorized as relating either to extrapolation (prediction of results outside the range of the observations) or to model definition (structure and parameter values).

TRICHLOROETHYLENE: PBPK models were developed for trichloroethylene (TCE) and its important metabolite trichloroacetic acid (TCA) in rats, mice, and humans (Volume II). The models for the rodent species (Fisher et al.,

1990) and the human model (Allen et al., 1990) were collaborative efforts between personnel at Clement International Corporation and Wright-Patterson Air Force Base. The human model is discussed in detail in Volume II, Part 1. The rodent and human models consisted of a PBPK model for TCE linked to a single compartment model for TCA. The link was defined in terms of TCE metabolism: a certain percentage of TCE metabolized by the P-450 system was converted to TCA and the remainder to products that were not considered.

Using the rodent and human TCE/TCA models, a risk assessment for TCE was conducted. Because TCA has been associated with liver tumors (Herren-Freund et al., 1987), and because the liver was assumed to be the only TCE-metabolizing organ, the assessment was based only on liver tumors occurring in mice. The dose surrogates examined were based on the amount of TCE metabolized per liver volume, the amount of TCA produced (again normalized for liver volume), and the area under the TCA concentration curve (TCA-AUC). These dose surrogates are specific to the liver or may be relevant to the estimation of liver cancer risk.

Three bioassays were examined (NCI, 1976; Bell et al., 1978; NTP, 1990). The bioassay data were used to estimate the continuous atmospheric concentrations of TCE and the drinking water concentrations (lifetime exposure in both cases) corresponding to a 10^{-6} (one in a million) level of extra risk in humans. The concentration estimates depended on the dose surrogate used to extrapolate across dose levels and across species. The estimates obtained from the three studies (one atmospheric and one drinking water concentration from each study) were as follows:

Dose Surrogate based on:	Air Conc. (ppt)	Water Conc. (µg/L)
TCE metabolized	320, 1200, 2700	7.6, 29, 62
TCA produced	69, 260, 570	1.6, 6.5, 13
TCA-AUC	9.7, 34, 80	0.23, 0.79, 1.9

Alternative approaches to the pharmacokinetic modeling of TCE and TCA are presented and discussed (Volume II, Part 2, Section B). These were primarily based on elaboration of the model for TCA kinetics; for example, one approach discussed involved inclusion of a liver compartment and an extrahepatic volume of distribution for TCA. Suggested alternative modeling approaches for modeling other metabolites of TCE that may also play a role in TCE [illegible]genesis are included.

Some discussion of peroxisome proliferation associated with TCE exposure is also presented (Volume II, Part 2, Section C). The manner in which such considerations could be built into a risk assessment for TCE is discussed, with emphasis on alternative dose-response modeling approaches. The degree of peroxisome proliferation might be used as an indicator of either an increase in mutation rates or an increase in cell turnover rates caused by TCE exposure. Inclusion of peroxisome proliferation represents a further refinement of the TCE model.

TETRACHLOROETHYLENE: Much of the discussion of tetrachloroethylene (perchloroethylene, PERC) pharmacokinetics is devoted to models that had been developed previously (Reitz and Nolan, 1986; Hattis et al., 1986) (Volume III, Part 1). Preliminary dose-response modeling of the cancer responses seen in rats and mice was completed, with the dose terms based on delivered dose (dose surrogate) estimates predicted by those models. That is, a derivation of risk

estimates was first completed without modification of the previously presented models and without extending them to consider TCA production and distribution. The dose surrogates examined in that case were based on PERC arterial blood and liver concentrations and on amount of PERC metabolized per liver volume.

Refinements and extensions of the PBPK models for PERC were also developed by Clement personnel for mice and humans (Volume III, Part 2). The refinements consisted of modifications to parameter estimates. The extension consisted of adding a submodel for TCA kinetics that therefore provided the ability to track the metabolite, TCA. The extension was implemented in the same manner as in the case of TCE: the model assumed that a certain percentage of the parent compound that is metabolized by the P-450 system was converted into TCA and the remainder into products that were not considered. The peer-reviewed literature was used as the source of data for developing and validating the models.

When the refined and extended mouse and human models were completed, they were used to estimate the atmospheric concentrations and drinking water concentrations that are associated with 10^{-6} (one in a million) extra risk, when exposure is continuous and lasts a lifetime. With the extended models, it was possible to determine delivered dose estimates based on the amount of PERC metabolized per liver volume, the amount of TCA produced (again normalized for liver volume), and the area under the TCA concentration curve (TCA-AUC). Using the NCI (1977a) and NTP (1986) bioassay results for male and female mice, the atmospheric concentrations and drinking water concentrations associated with a 10^{-6} extra risk were very consistent and were estimated to be as follows (on average):

Dose Surrogate based on:	Air Conc. (ppb)	Water Conc. (µg/L)
PERC metabolized	1.1	30
TCA produced	0.58	16
TCA-AUC	0.43	12

The uncertainties discussed in the general introduction (Volume I, Part 1) were considered for the PERC risk assessment (Volume III, Part 2, Section E) Some of those uncertainties had been addressed successfully with the extended PBPK models; however, some of the uncertainties associated with all risk assessments still could not be resolved.

METHYL CHLOROFORM: As in the cases of TCE and PERC, methyl chloroform (MC) produces TCA as one of its metabolites (Volume IV). PBPK models for MC/TCA were developed for mice and for humans. These models were identical in structure to the models developed for TCE and PERC. That is, they included a physiologically based model for MC coupled to a single-compartment model for TCA. The link between the MC and TCA submodels was through P-450 metabolism; a certain percentage of the MC so metabolized was converted to TCA.

The dose surrogates estimated from the mouse and humans models were used in the risk assessment of MC. The dose surrogates examined for MC were amount of TCA produced per liver volume and area under the TCA concentration curve. TCA-based dose surrogates were examined because of the known hepatocarcinogenic effects of TCA (Herren-Freund et al., 1987) and the relationship between TCA production and hepatocellular tumor response rates observed across the three chemicals, TCE, PERC, and MC (Volume IV, Section E).

The carcinogenicity bioassays of MC did not report a statistically significant increase in mouse liver tumors (NCI, 1977b; Quast et al., 1988).

The low rates of response in those studies were consistent with the TCA production estimates and the response rates observed after TCE or PERC exposure (Volume IV, Section E). Thus, the liver tumor response in male mice studied by NCI (1977b) was used with the TCA-based dose surrogates to estimate atmospheric concentrations and drinking water concentrations corresponding to a 10^{-6} extra lifetime cancer risk for humans:

Dose Surrogate based on:	Air Conc. (ppb)	Water Conc. (µg/L)
TCA produced	1.3	16
TCA-AUC	2.4	29

It was determined that the use of TCE or PERC bioassay results (in place of the MC results in male mice) would change the concentration estimates shown here only slightly.

VINYL CHLORIDE: The known human carcinogen, vinyl chloride (VC), was investigated. In particular, a PBPK model structure for VC kinetics that included conjugation of parent and metabolites with glutathione (GSH) was examined (Volume V). Parameter estimation and validation of such a model for rats were completed using data appearing in the peer-reviewed literature and data obtained from the laboratories at Wright-Patterson AFB.

Two sets of parameters for the rat model were examined. The predictions of experimental results provided by the two parameter sets were compared to each other and to predictions of simpler, alternative models. The simpler models did not incorporate a pathway for VC metabolism that represented conjugation with GSH. It was determined that the models which did include VC-

GSH conjugation appeared to provide a more accurate prediction of experimental results.

Using additional data from gas-uptake (closed chamber) studies conducted at Wright-Patterson AFB, the first steps were taken toward generalization of the rat model to other species. In fact, strain-specific results allowed the generalization to be initiated for various rat, mouse, and hamster strains that had been used for one or more cancer bioassays. Preliminary indications were that strain-specific models could be developed (i.e., fairly close agreement was obtained between strain-specific model predictions and strain-specific gas uptake experiment results). Further refinement of parameter estimates is suggested (Volume V, Part 2).

SENSITIVITY/UNCERTAINTY ANALYSES: Analyses of the sensitivity of PBPK models to changes in the input parameters were conducted. These analyses were completed for three parameter sets (representing three different species) and for a variety of exposure patterns. The degree of sensitivity of the model[1] to the parameter values depended on the dose surrogate considered and, to some extent, on the route of exposure and species. The sensitivity analysis provided a basis for determining which parameters are likely to have the greatest impact on PBPK model output (dose surrogate estimates).

In addition, an uncertainty analysis was conducted for the same model. The uncertainty analysis differed from the sensitivity analysis in that variability of parameter values was estimated from the literature and

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[1]The model was a relatively simple, four compartment model with saturable metabolism occurring in one of those compartments (the liver). The model and the parameter sets were based on the PBPK model that had been proposed for PERC with parameters estimated for rats, mice, and humans.

distributions describing that variability (or uncertainty) were derived. Moreover, instead of varying one parameter at a time, all values for all the parameters were sampled from their respective distributions to determine the overall impact of parameter uncertainty on dose surrogate estimation and on the estimation of risks. For risk estimate uncertainty, the response rates (numbers of animals found to have a tumor relative to the total number of animals being studied) were also allowed to vary.

The results of such an uncertainty analysis are distributions of dose surrogate values and/or risk estimates. The distributions reflect both the sensitivity of the model to the parameters and the degree to which the values of the parameters are uncertain. Such distributions were derived for the simple PERC model applied in the context of a bioassay with liver carcinoma responses in female mice as the endpoint (NTP, 1986).

Also presented is an approach for determining the importance of individual parameters (or sets of interrelated parameters) to the total uncertainty represented by the distribution of dose surrogate or risk estimates. That approach involved varying only one parameter (or set of parameters) at a time, but unlike the sensitivity analysis, the variation was defined in accordance with the uncertainty distribution for the parameter (as opposed to an arbitrary increase or decrease in the parameter value). Variability in the output observed when each parameter was varied by itself was compared to the variability observed when all parameters varied. The percentage of the total variability induced when a single parameter is varied is an indication of the importance of that parameter, given the current state of knowledge about the values it can assume.

For the PERC model under consideration, it was found that risk estimates derived when one uses a dose surrogate based on the amount of PERC metabolized (per liver volume) spanned a range of about seven orders of magnitude, when all parameters were allowed to vary. The variation was less extreme when other dose surrogates were used. In any case, the variability can be used to make probability statements about individuals exposed to a chemical and at risk of developing cancer.

The contribution of individual parameters was found to vary depending on the choice of dose surrogate. For a dose surrogate based on the arterial concentration of PERC, the blood/air partition coefficients contributed greatly to the risk estimate distribution, accounting for 26 to 32% of the overall uncertainty in the risk estimates.[2] When risks were derived using liver concentration as the basis for dose surrogate definition, variability of the human liver/air partition coefficient accounted for 88% of the total risk estimate uncertainty. This work is presented in Volume VI, which includes a published paper by Farrar et al. (1989).

UNCERTAINTY SOFTWARE: The final volume (Volume VII) of this report contains the documentation for the software that was developed to facilitate the type of uncertainty analysis discussed above. The software (PBPK_SIM) allows the user to define the distributions for representing model parameter uncertainties, to run a number of simulations as specified by the user (where the parameter values vary from simulation to simulation), and to statistically

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[2]The 26% value was obtained when the mouse blood/air partition coefficient was allowed to vary; the 32% value was obtained when the human blood/air partition coefficient was allowed to vary. When both were allowed to vary, about 81% of the total variability in the risk estimates was accounted for.

describe the variability of the resulting output. Any PBPK model can be used with the software, if the model is defined in accordance with some specific rules. The model must be defined, through a CSL file, in the format specified by ACSL[3] and must conform to certain other rules related to the labeling of parameters or groups of parameters. The labeling requirements do not detract from the flexibility available through ACSL.

Another important feature of the PBPK_SIM software is that it links the pharmacokinetic simulations with dose-response modeling. Several versions of the multistage model (Howe et al., 1986) can be selected for cancer dose-response modeling. The user can request that distributions of risk estimates be generated. For each set of test species and human dose surrogate estimates (corresponding to one of the simulations requested) a risk estimate will be derived. This is accomplished by assuming that animals and humans are equally sensitive to the carcinogenic effects of the compound under consideration when doses are expressed in terms of the dose surrogate(s) specified.

[3]ACSL - Advanced Continuous Simulation Language, Mitchell and Gauthier Associates, Inc., Concord, MA. ACSL was used as the basis for PBPK_SIM; that is, ACSL is the system used to implement and solve the system of differential and algebraic equations that define a PBPK model.

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INTRODUCTION

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A. BACKGROUND

This report presents the results of a multiyear study designed to examine pharmacokinetics and pharmacokinetic modeling in the context of risk assessment. Those efforts have included the following types of activities: development of physiologically based pharmacokinetic (PBPK) models for selected chemicals, application of the results of the pharmacokinetic models (dose surrogate estimates) to the estimation of human cancer risk from exposure to these selected compounds, investigation of uncertainty and sensitivity associated with pharmacokinetic model parameters, and development of software designed to automate the process of considering uncertainties associated with PBPK-assisted risk assessment. Each of these efforts is discussed in detail in Volumes II through VII of this document.

The goals of this project included the development and application of procedures for incorporating PBPK modeling into risk assessment. The procedures developed and applied were intended to enhance the credibility of risk assessment either by increasing its predictive ability or by demonstrating how to deal with uncertainties associated with risk assessment. Those goals have been pursued primarily through specific examples applied to volatile organic chemicals.

Outlined here are some of the general issues associated with the use of PBPK modeling and pharmacokinetic data for risk assessment. Presentation of these issues, coupled with the applications presented in the other volumes of this report, illustrates the utility of pharmacokinetics for risk assessment.

B. DEVELOPMENT OF PBPK MODELS

A basic approach was developed during the course of this project that appears to be feasible for PBPK model development and application to risk assessment problems for any compound. That approach is summarized as follows.

A relatively simple PBPK model is selected for initial examination and comparison with quantitative pharmacokinetic data. Generally, the data required have been available in the literature.[1] Sometimes, unpublished experimental results (such as those obtained from Harry G. Armstrong Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base) were available.

The comparison of model predictions and experimental results is iterated through several versions of the model. For each iteration, the model is refined, either by modifying parameter values or by modifying the model structure. The latter may entail estimation of the values of additional parameters. The iteration is stopped when model predictions are deemed to adequately predict the observed experimental results.

This model development procedure is completed for all species of interest. When data are limited for a certain species, scaling (allometry) may be employed to aid the parameter estimation. It may be the case that the development of validated PBPK models for a number of chemicals (especially those that belong to particular classes, such as volatile organics) can be the basis for uncovering patterns of parameter values (across chemicals or

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[1]For some compounds, pharmacokinetic data may be more difficult to obtain from published reports. It is hoped that an emphasis on pharmacokinetic modeling for risk assessment will have an impact by increasing the amount of effort devoted to pharmacokinetic analyses, which in turn would encourage even greater use or pharmacokinetic modeling.

species) and therefore provide information for use in cases where the data are limited.

C. USE OF PBPK MODEL OUTPUT

Once the species-specific PBPK models are developed and validated, they are used to estimate values for dose surrogates, i.e., dose metrics based on the amount or concentration of a chemical at particular sites in the body. The choice of the site(s) and the dose surrogate(s) depends on the particular application. Factors that influence that choice include the site of toxicity, the type of toxicity, and the presumed mechanism producing that toxicity (Gillette, 1987). Dose surrogates are used (in place of administered or experimental doses) as input for dose-response (e.g., multistage) modeling.

1. Selection of Dose Surrogates

The doses typically used in regulatory risk assessments are the administered doses expressed in terms of some standard units, such as mg/kg body weight/day. Humans are assumed to be equally sensitive in terms of cancer risk, when this dose is expressed in the same unit for both species. Use of the administered dose, however, does not account for all the differences between species or routes of administration that determine the amount of toxicant at tissues susceptible to carcinogenic attack. The same applied or administered dose will produce a different delivered (surrogate) dose at the target site, and possibly different effects, depending on the bioavailability of the toxicant, which may be species- or route-specific. PBPK

modeling, by describing the kinetics of this bioavailability, provides the means by which dose surrogates at target sites can be estimated.

A surrogate dose is a measure of some substance, usually at a particular site in the body, that can be used in dose-response estimation. The tissues of greatest concern in the risk assessment context are those that are the sites of carcinogenic action; therefore, it is of interest to estimate surrogate doses associated with those sites. In risk assessment extrapolations utilizing pharmacokinetic data, it is assumed that a particular level of a surrogate dose at a specific target tissue achieved by one route of exposure in a particular species will have the same biological effect, that is equivalent risk, as an equal level of the surrogate dose achieved by another route or in another species.

The surrogate is chosen on the basis of some known or supposed relationship between that surrogate and the response of interest. The substances of potential usefulness are the parent compounds (administered to test species or encountered by humans) or metabolic products of the parent. The particular substance used as the basis for surrogate dose estimation depends on the available data describing the toxicity of the parent and its metabolites.

The surrogate doses used in this investigation were based on amounts per volume of tissue, i.e., concentrations. If one assumes uniform distribution of chemicals within a compartment, then a concentration (amount per volume) should be proportional to the amount of toxicant <u>per cell</u>. That is the desired endpoint for PBPK modeling. If the (somewhat artificial) distinction is made between pharmacokinetics and pharmacodynamics, then the intracellular events that occur after delivery of the toxicant to the target tissue are not

within the scope of PBPK modeling. However, the estimation of the amount of toxicant per cell or the proportional quantity, tissue concentration, should provide the starting point for modeling the pharmacodynamic events.

The specific assumption underlying the use of concentration-based surrogate doses in existing dose-response models is that the same amount of toxicant in two different cells will produce the same level of an effect (perhaps in a probabilistic sense). This assumption may be made whether or not the two cells come from the same organ or even from the same species. This is the basis of the extrapolation from animals to humans: the probability of response in human cells is the same as that for animal cells given equal amounts of toxicant in the cells. The current regulatory approach using administered dose includes a corresponding assumption: the probability of response for a human as a whole is the same as that for an animal if the daily amount of parent compound encountered (scaled accordingly, for example, according to surface area or body weight) is the same as encountered by the animal.

It is assumed that the greater the amount of toxicant per cell, the greater the probability of toxic response. It may also be appropriate to assume that the longer the toxicant is present in the cells, the greater the probability of response. Examples of surrogate doses that combine these properties, i.e., that are increasing functions of concentration and time, are areas under concentration curves. Surrogate doses used in this investigation were expressed as areas under such curves.

Note that for short-lived, reactive metabolites whose exact identity may be unknown, it may not be possible to directly measure the area under the concentration curve. This is due to the fact that their fate and the rate of

their breakdown may be unknown. Andersen (1981) has shown that under certain conditions the amount of parent metabolized divided by the volume of the metabolizing organ is proportional to the area under the metabolite concentration curve. When such short-lived metabolites were of interest, the dose surrogates used in this investigation were amounts of parent metabolized per volume of tissue, termed the virtual concentrations of the metabolite.

2. Use of Dose Surrogates for Dose-Response Modeling

Once dose surrogates presumed to be potentially related to the induction of toxicity (cancer) are selected, the application of those surrogates to dose-response modeling is relatively straight-forward. The process is completely described by the following steps:

1) Using the species-specific and chemical-specific parameters corresponding to the test species exhibiting the toxicity, run the PBPK model to determine values of the relevant surrogate doses for each dose group;

2) Using the parameters corresponding to humans, the concentration x of the chemical, and the exposure route for which risk estimates are desired, run the PBPK model to obtain the values of the dose surrogates corresponding to that exposure.

3) Run the dose-response model with the input doses given by the surrogate doses determined in (1), the response rates as given in the bioassays, and the doses for which risk estimates are desired given by the human surrogate doses determined in (2).

The results from step (3) are the risk estimates desired.

The values of the selected dose surrogates are used as the dose terms for dose-response modeling. This can be done no matter what dose-response model is used. The assumptions underlying this use of the dose surrogate estimates are that the PBPK models predict their values correctly for all exposure levels (a low-dose extrapolation assumption in most risk assessments)

and that human and nonhuman species are equally sensitive (have the same probability of response) when the dose surrogate values are the same.

In the investigations discussed in this report the dose-response model used was the multistage model (Howe et al., 1986). The procedure described above can be used with any dose-response model, however.

No matter what model is used, an assumption must be made about the equivalence of humans and other animals (e.g., those tested in carcinogenicity bioassays) with respect to the probability of response in the presence of the carcinogen. The assumption can be stated as follows: the probability of response in one animal is the same as that in other animals (within a specific set of animals) when the appropriate dose surrogate values (resulting from exposure) are the same for those animals. The definition of response may vary depending on the dose-response model selected.

D. UNCERTAINTY

The importance of uncertainties in risk assessment cannot be overemphasized. Goals of any proposed refinement of risk assessment methodology should include the reduction of uncertainty or elucidation of means to handle uncertainty. Both are goals of proposed refinements based on the incorporation of PBPK modeling into risk assessment. Preliminary considerations concerning uncertainties in risk assessment are presented here.

One of the most serious uncertainties associated with risk assessment practice is uncertainty with respect to the relevance of the models used. This uncertainty relates to cause and effect relationships. There may be several alternative methods (models) for relating a cause (e.g., exposure to a

carcinogen) to an effect (e.g., probability of cancer). The selection of one model from among the alternatives leads to uncertainty.

This is a difficult aspect of uncertainty to quantify or to express statistically, e.g., in the context of confidence limits on estimates. It is an uncertainty that may be more a matter of agreement (or the degree of disagreement) among knowledgeable scientists than is the case for other uncertainties.

With respect to uncertainty associated with model selection, the incorporation of PBPK modeling into risk assessment would probably be recognized as reducing the uncertainty in the risk assessment process. Alternative PBPK models may exist, and they may represent different relationships between exposure and delivery of toxicant. In theory, however, it should be possible to differentiate among alternative PBPK models through experimentation and comparison of model predictions with experimental observations. This is not always the case with dose-response models where it may be impossible to differentiate between models (differing primarily with respect to low-dose risk estimates) through the comparison of predictions to observations. Some of the experiments that theoretically could differentiate between such alternative dose-response models may be impossible logistically. An interesting line of investigation for risk assessment relates to the design of experiments (e.g., *in vitro* tests) that are relevant to dose-response issues.

Moreover, by explicitly considering the fate of a chemical after it is encountered by an animal, and by accounting for differences among species in the quantitative aspects of that fate, it appears that PBPK may be able to reduce uncertainties related to other cause and effect relationships. That

is, use of delivered dose estimates eliminates uncertainties and biases associated with use of applied (administered) doses for determining relationships between dose and toxic response. Use of administered dose does not incorporate information on pharmacokinetic processes that affect delivery at various doses nor does it incorporate species differences in those processes. Thus, the underlying relationship between (delivered) dose and response can be obscured if administered doses are used as the basis for investigating that relationship.

Other uncertainties that have been considered can be categorized as being either model uncertainties or extrapolative uncertainties.

1. Model Uncertainties

PBPK modeling is a technique that allows the estimation of the amount of parent or metabolite in the various tissues of the body over time. As with all models, especially those used in the biological sciences, PBPK models have uncertainties associated with them, that is, the predictions of the models are uncertain. Because model predictions depend on specified values for the model parameters, any error in those values may produce inaccuracies in the predictions. Parameter values may be in error for two reasons: variability and uncertainty.

PBPK model parameters have an inherent variability that is characteristic of heterogeneous populations. For example, not all mice have the same volume of liver, cardiac output, or ventilation rate. Some differences can be accounted for by scaling such parameters according to body weight but these are only the gross differences expected in animals of different sizes. The scaling factors themselves are estimated; they are based on average values for

groups of animals so they are themselves subject to the variability within populations. This inherent variability cannot be eliminated. At best, it should be possible in principle to derive a distribution for representing the variability of a parameter (or set of interrelated parameters); this is the basis for the uncertainty analyses discussed in Volume VI and of the software discussed in Volume VII. Such considerations lead to distributions of risk estimates as the output of a risk assessment. At the very least, one must be cautious when interpreting risk assessments that present a single risk estimate and always keep in mind inter-individual variability in heterogeneous populations.

Confounding the problem of inter-individual variation is uncertainty about the "true" values for a parameter. In the context of the distributions representing variation within a population, this uncertainty may be restated as uncertainty about the value of the mean of the distribution. Such uncertainty appears to be associated with measurement errors or differences in measurements obtained from different techniques or different laboratories.

This type of uncertainty may be resolvable through more and better measurement. Of particular interest are measurements obtained from the same animals used for toxicity testing, the results of which are used to investigate dose-response relationships, for example. Then one would have the values or range of values that are most pertinent to estimating delivery of dose to the target sites at which toxicity is measured and reported. This is especially valuable if the act of administering the dose is liable to affect the animals in such a way that values for the parameters change (e.g., if inhalation exposures cause the animals to alter their ventilation).

Some parameter estimates may have greater uncertainty associated with them than do others. This may be largely a matter of gaps in the data and methods that are employed to compensate for those gaps. Consider, for example, partition coefficient estimates and methods used to estimate those for tissues and species lacking direct measures. For tetrachloroethylene (PERC, see Volume III) tissue/air partition coefficients were not available for human tissues other than blood. Reitz and Nolan (1986) estimated those coefficients by an averaging rat and mouse values. Hattis et al. (1986) used a regression technique to estimate human partition coefficients. Another approach was suggested by Andersen et al. (1987) in their PBPK modeling of methylene chloride; they assumed that tissue/air coefficients in humans are the same as in rats. Thus, three different sets of assumptions have been used to derive human partition coefficients.

Additional uncertainties are associated with compartments that are composed of a variety of tissues or organs. Partition coefficients for those compartments (richly perfused and slowly perfused tissues, for example, which are composed of various organs), are often estimated on the basis of single samples. Such estimation can be inaccurate because of the complex nature of the tissues that make up the compartments. Population heterogeneity also affects these estimates: if the relative contribution of the organs and tissues comprising a compartment varies over the population, then a single value for a partition coefficient may be inadequate. This would be the case even if the partition coefficient estimate is derived, for example, by a weighted average of partition coefficients for the tissues comprising the compartment and those tissue-specific coefficients did not vary.

Another uncertainty arises when values of parameters are estimated by means that require estimates of other parameters. Estimation of metabolic constants is a particularly relevant example. All the *in vivo* estimation techniques (e.g., gas uptake methods) require estimates of the other parameters. Expressed in another way, metabolic constant estimation is dependent on (is a function of) the values of the other model parameters. *In vitro* approaches may not depend on estimates of other parameters, but the extrapolation to the *in vivo* situation adds another level of uncertainty, as does the dependence of the *in vivo* techniques on the already uncertain estimation of physiological and physicochemical parameters.

2. Extrapolative Uncertainties

The uncertainties discussed above, those that we have labeled model uncertainties, relate to the estimation of surrogate dose values. The uncertainties that remain are more pertinent to the question of what one should do with the dose surrogate estimates once they have been derived. These uncertainties are frequently uncertainties at a more fundamental level. That is, they often involve issues that go beyond pharmacokinetics and into areas for which our base of knowledge is much more limited.

Dose Surrogates: The most obvious source of uncertainty in the specification of surrogate doses is in the choice of the compound that is to form the basis for the surrogate. For many compounds, especially the volatile organics, it has been argued that metabolites, and not the parent compounds, are responsible for the carcinogenicity associated with exposure to the parent. As presented in the subsequent volumes of this document, however, the type of metabolite thought to be the ultimate carcinogen is not the same for

all volatile organics. It is proposed below that a reactive short-lived intermediate metabolite is responsible for the carcinogenicity of vinyl chloride (Volume V) but the evidence for trichloroethylene and tetrachloroethylene (Volumes II and III) suggests that a persistent product, trichloroacetic acid, is responsible (at least in part) for the tumorigenicity of those compounds.

Thus the identification of an appropriate or representative compound for use as the basis for defining a relevant dose surrogate, one that can be estimated by a PBPK model and used in dose-response modeling, may be problematic. Issues such as biological reactivity or effects that may indirectly affect cancer causing processes (cf. the discussion of peroxisome proliferation in Volume II) must enter into such a decision.

The choice of certain compounds as the basis for dose surrogate definition carries with it certain assumptions. If, for example, it is assumed that short-lived metabolites are the substances responsible for carcinogenicity, dose surrogates based on those metabolites are appropriate for use only in organs where metabolism can take place. Such compounds, by assumption, are not transported outside the metabolizing tissue. Thus, it may not be appropriate to use the same dose surrogate for all cancer responses.

Assumptions associated with the selection of a dose surrogate also extend to the dose-response modeling side of risk assessment. Certain surrogates may only be related to specific steps in the overall process leading from normal to cancer cells. Short-lived reactive compounds, for example, may only be associated with interactions with DNA leading to mutations. It may not be appropriate in that case to relate such a surrogate to cell proliferation rates. Use of the linearized multistage model (Howe et

al., 1986) for which specific steps are not identified, obscures this uncertainty. An argument was presented above in favor of using chemical concentrations as the basis for dose surrogate definition. However, it is one that may have to be considered if a risk assessment is to use other dose-response models that differentiate between mutagenic effects and proliferative effects (e.g., the two stage model of Moolgavkar and Knudson, 1981).

In some cases it may be difficult to define a surrogate that can be estimated by a PBPK model. The induction of some tumors has been related to some sort of "stress" induced in the corresponding tissue in response to the presence of compound (e.g., tetrachloroethylene and the kidney tumors observed in rats; NTP, 1986). The nature of the stress may be unknown, and, in any case, may be difficult to represent by a variable estimable by a PBPK model.

There exist other difficulties relating to the definition of appropriate dose surrogates. An argument was presented above in favor of using chemical concentrations as the basis for dose surrogate definition. However, given the lack of knowledge concerning relevant intracellular events, there is uncertainty associated with selection of concentration-based estimates. It is not inconceivable that concentrations above a certain "threshold" value are the relevant measure of dose. This may indeed be a plausible scenario if something other than direct interaction with macromolecules (e.g., cytotoxicity) is the underlying mechanism of carcinogenicity. Similar considerations may apply if a compound acts to increase membrane permeability or in some other way results in the facilitation of cancer-causing events not strictly tied to the compound itself. Some mechanisms relating to cell proliferation may also fall into this category.

Other plausible surrogate doses could be defined and form the basis for risk estimation. These other possibilities have not been explored in this investigation.

Cell Differences: The major assumption underlying any risk assessment based on nonhuman experimental results is that there exists some dose surrogate that can be related to some process in such a way that that relationship is the same for all species (or some group of species relevant to the risk estimation). This assumption may be restated: the probability of response in one species is the same as that in another species when the values of the appropriate dose surrogate are the same in the two species. In this form, it is apparent that this is a scale-up assumption. The uncertainty relates to finding an appropriate dose surrogate and an appropriate definition of response.

At present it is not clear how any PBPK-estimated variable should be scaled to yield the appropriate dose surrogate. Such scaling should account for cell differences and other species differences, including differences in inherent susceptibility, in longevity, in repair mechanisms, and in total number of cells. Some of these differences are present within one species (e.g., repair capacity may vary from one tissue to another for any given species). If a suitable compound for use as the basis for dose surrogate definition could be agreed upon, then a plausible empirical investigation would be an examination of the species (and tissue-to-tissue) scaling that most successfully accomplishes extrapolations. This is an issue that deserves further attention especially in light of possible species differences that are not accounted for by pharmacokinetic analysis. The uncertainty associated with this issue may be significant.

Short-lived Metabolites: Another example of possible species differences illustrates the uncertainty related to species extrapolation. For short-lived reactive intermediates, it is not possible to directly estimate area under the concentration curve. A dose surrogate used in place of the direct estimate is the amount of parent metabolized per volume of metabolizing tissue. Andersen (1981) has shown this surrogate to be proportional to the direct estimate, under reasonable conditions.

For cross-species extrapolation of risks that are based on this dose surrogate, it is assumed that the proportionality of the surrogate to the true value is the same across species. In fact, that assumption is crucial for use of that surrogate. If the proportionality differs across species, then the risk estimates will change by a factor equal to the ratio of the proportionality constants. This may be a significant source of uncertainty; proportionality constants for two species will be the same only if the rate at which the reactive metabolite reacts with the receptor, which ultimately causes the cancer, compared to the rates of competing pathways, is the same for those two species.

The uncertainty mentioned in the preceding paragraph relates not to the choice of dose surrogate, but rather to the estimation of values for the surrogate that are used for extrapolation. Whenever the reactive metabolite is present for a longer period of time in one species than in another species, the probability of toxicological response is likely to differ between the two species. Consequently, whenever a dose surrogate is based on the rate of disappearance of the parent, rather than on the appearance and disappearance of the metabolite itself, this uncertainty will remain.

Meaning of Extrapolated Risks: A further difficulty related to the choice and use of dose surrogates relates to the meaning of risks extrapolated from rodents to humans. Consider the liver tumors produced in mice exposed to tetrachloroethylene and a dose surrogate based on the concentration of a metabolite (cf. Volume III). When the value of the dose surrogate (in a specified organ) corresponding to a specified human exposure scenario is estimated and then risk is based on that estimate and the observed liver responses in mice, what is it that is being estimated? Is it the risk of liver cancer in humans exposed in the manner specified? If it is intended to be a general estimate of cancer risk, i.e., without reference to specific sites of occurrence, then what relevance does a liver metabolite concentration have for an estimate of such a general effect.

When using administered doses, as was done for most of the risk assessments that have been completed over the years, the specific tumors observed in the rodents tested were of only secondary importance to the estimation of risk. The reasoning can be summarized as follows: mice given administered dose X have probability Y of getting the type of tumor that mice get in response to the chemical; if humans are as sensitive as mice, then their probability of getting the type of tumors that humans get in response to the chemical is Y when they are exposed to amount X. (The policy adopted by regulatory agencies has been that it is prudent or health-protective to assume that humans are as sensitive as the animals tested.) Organ-specific surrogates now available because of PBPK modeling are not amenable to this type of reasoning.

One potential interpretation of risk estimates based on dose surrogates is that it is not the organ that is important but rather the concentration

curve itself. In that case, the extrapolation of risk could be conducted as follows: the mice have a probability Y of getting liver cancer when the area under the liver concentration curve is X; then the probability that humans will get the type of tumor that they get is Y when the area under the concentration curve in the organ where that tumor appears is X. For an example consider methylene chloride (DCM). The epidemiological evidence sheds little light on the site of action of DCM-induced carcinogenicity in humans, but the results of a study by Hearne et al. (1986) may indicate that the pancreas is the site of carcinogenic attack in humans. If that is the case, then the procedure that would be consistent with the interpretation just described would be to estimate the area under the pancreas concentration curve corresponding to the exposure scenario of interest and use that as the dose for which risk estimates are desired no matter which mouse or rat response is being modeled by the dose-response model. This has not been done in this investigation.

The preceding discussion highlights a potential difficulty in any extrapolation scheme. It is frequently the case that the only evidence of carcinogenicity of a chemical comes from animal tests. In fact, in the best of circumstances, the bioassays and other tests relevant to carcinogenicity should be used in a predictive manner. That is, one would hope to be able to predict the outcome of human exposure to the chemical in question before such exposure occurs; risk assessment has as its goal the minimizing of harmful exposures. In such situations, the site of action in humans is unknown. Hence, it is not possible to specify the site at which concentrations should be estimated. A general surrogate such as concentration in the arterial blood may then be most convenient as a measure of "whole body" exposure not tied to

specific organs. A profile of the arterial blood concentration provides a measure of dose that is the same for most organs; i.e., the delivery of dose to most organs is the same because arterial blood is the only (or primary) route of delivery. The compound selected for use in calculating arterial blood concentration should be chosen in accordance with the considerations given above.

Thus, there is considerable uncertainty regarding the use of whatever dose surrogate estimates are obtained. At present, it may only be possible to minimize them in whatever risk assessments are performed. The explicit consideration of these uncertainties is warranted. Pharmacokinetic information has made possible the recognition of some of the uncertainties. In fact, PBPK modeling, by providing more specific estimates (with respect to compound and site), also provides the tools for addressing uncertainties.

E. DOCUMENT ORGANIZATION

This document is organized into seven volumes. Aside from this first volume, each volume is devoted to one of the tasks that was completed in the course of this investigation. Volumes II through V discuss four volatile organic chemicals for which PBPK and/or dose-response modeling work has been done. The chemicals are trichloroethylene (Volume II), tetrachloroethylene (III), methyl chloroform (IV), and vinyl chloride (V). Each of these volumes presents the data used and the results obtained in relation to PBPK modeling. For trichloroethylene, tetrachloroethylene, and methyl chloroform, risk estimates based on mouse liver tumor responses are also presented.

Volume VI presents a description of sensitivity analyses that were completed for a simple PBPK model and associated risk assessment. Volume VII is the documentation for a software package designed to do the type of analyses discussed in Volume VI. The software links PBPK models and their output to dose-response models. The documentation describes the statistical distributions for defining the variability of parameter estimates and the simulations that can be performed using such distributions.

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